Kidney International, Vol. 46 (1994), pp. 1459–1472

Abstracts

Dutch Society of Nephrology
48th Scientific Meeting
'S-Gravenhage
October 30, 1993

Renal ischemia contributes to renal damage in rat model of anti-myeloperoxidase associated necrotizing crescentic glomerulonephritis (NCGN). *E. Brouwer, P.A. Klok, M.G. Huitema, J.J. Weening, and C.G.M. Kallenberg, Depts. of Clinical Immunology and Pathology, Academic Medical Centre, and University Hospital Groningen, The Netherlands.* Human anti-myeloperoxidase (MPO) associated NCGN is characterized by crescent formation and glomerular and interstitial infiltration with inflammatory cells. Focal fibrinoid necrosis of capillary loops is present at a very early stage in the development of NCGN, indicating that focal endothelial cell damage and GBM necrosis, possibly due to focal ischemia, are prerequisites for the development of NCGN in the presence of anti-MPO antibodies. In a recently developed model of anti-MPO associated NCGN we showed that anti-MPO alone is not sufficient for the induction of NCGN, but that MPO, lytic enzymes, and oxygen radicals need to be localized along the GBM as well. Since ischemia triggers endothelial cells to produce oxygen radicals, we tested whether renal ischemia plays a role in the induction of anti-MPO associated NCGN. Ischemia was induced in MPO-immunized rats ($N = 13$) perfused with a lysosomal extract containing MPO, HLE, and Pr3, by clamping the left renal artery for 20 minutes after perfusion (group I). The control groups consisted of MPO immunized rats ($N = 7$) perfused with Pr3, MPO, and HLE which were not clamped (group II), and control immunized rats ($N = 6$) perfused with the lysosomal extract containing MPO, HLE, and Pr3, and clamped for 20 minutes (group III). Rats were sacrificed at 24 hours, 4 days, and 10 days after perfusion. By lightmicroscopy we found necrosis, crescent formation and infiltration of inflammatory cells as shown in the table in group I, but not in groups II and III. MPO, immunoglobulin (Ig), and complement (C3) deposition and the numbers of infiltrated neutrophils (PMN) and monocytes (MO) were quantified by immunohistochemistry.

Groups	Staining	24 hours	4 days	10 days
I	MO	2.8 ± 1.1	3.1 ± 0.8	7.1 ± 4.3
II	MO	1.5 ± 0.3	1.4 ± 0.1	0.2 ± 0.2
III	MO	0.3 ± 0.2	0.3 ± 0.0	0.6 ± 0.3
I	PMN	2.6 ± 0.5	3.1 ± 1.5	1.6 ± 0.5
II	PMN	1.7 ± 0.1	0.6 ± 0.1	0.4 ± 0.2
III	PMN	0.7 ± 0.1	0.8 ± 0.2	0.5 ± 0.4

Groups	Staining	24 hours	4 days	10 days
I	MPO	+++	++	±
II	MPO	+	−	−
III	MPO	−	−	−
I	IgG/C3	+±/++±	+±/++	+±/+
II	IgG/C3	++/++	+/+	±/±
III	IgG/C3	−/−	−/−	−/−

We conclude that renal ischemia may indeed play a role in the induction of anti-MPO associated NCGN.

The renal effect of adenosine infusion depends on the route of administration. *R. Fransen and H.A. Koomans, Utrecht University Hospital, Dept. of Nephrology, Utrecht, The Netherlands.* Systemic infusion of adenosine (ADO) causes anti-diuresis in the rat, whereas intrarenal infusion has been reported to cause diuresis. These opposite effects have not been demonstrated within the same animal. Furthermore, the tubular location of the diuretic effect has not been investigated. A small catheter was advanced into the left renal artery of male Sprague Dawley rats and saline with different ADO concentrations was infused at a constant rate. Next, the catheter was repositioned into the thoracic aorta, above both renal arteries and, after saline, ADO (50 μg/min) was infused.

	U-flow *μl/min*		GFR *ml/min*	
	Right	Left	Right	Left
Intra-renal ADO				
0 μg/min	4.8 ± 0.7	5.2 ± 2.4	1.34 ± 0.04	1.32 ± 0.07
5 μg/min	4.8 ± 0.2	12.5 ± 2.1[a]	1.31 ± 0.06	1.42 ± 0.14
10 μg/min	5.1 ± 0.9	13.7 ± 3.7[a]	1.34 ± 0.10	1.24 ± 0.12
50 μg/min	5.8 ± 1.9	12.5 ± 3.1[a]	1.34 ± 0.16	1.16 ± 0.06
Aorta ADO				
0 μg/min	5.1 ± 1.0	5.5 ± 1.5	1.36 ± 0.16	1.17 ± 0.06
50 μg/min	2.9 ± 0.3[a]	3.1 ± 1.0[a]	0.92 ± 0.01[a]	0.92 ± 0.04[a]

Data are mean ± SE; [a] $P < 0.05$ vs. infusion of saline without ADO (ANOVA)

Blood pressure and filtration fraction did not change during the study. Intrarenal infusion caused diuresis and natriuresis (not shown) without a change in GFR, suggesting a direct tubular effect. ADO infusion in the aorta of the same animal resulted in antidiuresis, and a reduction in GFR. Intrarenal ADO infusion, below 5 μg/min, had no effect on urine flow or GFR. Thus, it is unlikely that the different response to the administration routes depends on different ADO concentrations reaching the kidney. To assess the effect of intra-renal ADO, we performed micropuncture in rats infused intra-renally with saline or 10 μg/min ADO. Urine flow increased as in the clearance study. No significant change in absolute or fractional delivery of water or sodium up to the early distal tubule was observed. We conclude that the intrarenal administration of ADO increases renal excretion of water and sodium, probably by a direct tubular effect. This effect is not located in the proximal tubule or loop of Henle of superficial tubules. Systemic ADO infusion causes antidiuresis, partly by reducing GFR.

ATP-induced inhibition of calcium transport in primary cultures of rabbit cortical collecting system is mediated by P_2-purinergic receptors. *R.J.M. Bindels, A. Hartog, and C.H. van Os, Department of Physiology, University of Nijmegen, The Netherlands.* Active transcellular Ca^{2+} transport in the rabbit kidney collecting system has been shown to be inhibited by pharmacological activation of protein kinase C (PKC). Until now, a physiological agonist of this regulatory pathway has not been identified. Therefore, the role of extracellular ATP in regulating transcellular Ca^{2+} transport as possible activator of PKC was investigated. Cells from the rabbit cortical collecting system were isolated by immunodissection with Mab R2G9 and subsequently cultured on permeable filters or on glass coverslips to determine transcellular Ca^{2+} transport and intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), respectively. ATP (10^{-7} to 10^{-3} M) reduced

active transcellular Ca^{2+} transport dose-dependently (IC_{50} = 3 μM), with a maximal inhibition of 43%. At 10^{-4} M, ADP had no effect on transcellular Ca^{2+} transport. On the contrary, AMP and adenosine stimulated Ca^{2+} transport rate by 118 ± 3% ($P < 0.05$). This order of potency suggests that ATP-induced inhibition is mediated by P_2 purinergic receptors. At the cellular level, ATP (10^{-7} to 10^{-3} M) caused a rapid transient increase in $[Ca^{2+}]_i$ which returned to a slightly elevated sustained level. The peak value of the $[Ca^{2+}]_i$ transient increased dose-dependently with an apparent K_d of 1 μM ATP. In the absence of extracellular Ca^{2+}, a similar Ca^{2+} peak was induced by ATP indicative for Ca^{2+} release from intracellular stores, but the sustained response was abolished. Loading the cells with the Ca^{2+} chelator BAPTA completely prevented the ATP-induced Ca^{2+} transients, without diminishing the inhibition of transcellular Ca^{2+} transport. The protein kinase C activator, diacylglycerol (DAG), mimicked the inhibitory effect of ATP on transcellular Ca^{2+} transport. In conclusion, extracellular ATP presumably binds to P_2 receptors which leads to activation of phospholipase C. Only activation of PKC by DAG results in inhibition of transcellular Ca^{2+} transport, while the IP_3-induced Ca^{2+} release and activation of a Ca^{2+} entry mechanism does not affect transcellular Ca^{2+} transport.

Cardiac renin is kidney-derived. *A.H.J. Danser, P.J.J. Admiraal, F.H.M. Derkx, J.M.J. Lamers, P.D. Verdouw, P.R. Saxena, and M.A.D.H. Schalekamp, Depts. of Pharmacology, Biochemistry and Internal Medicine I and Thoraxcenter, Erasmus University, Rotterdam, The Netherlands.* The heart is capable of generating and releasing angiotensin I (Ang I) and II (Ang II), at least when renin is present in the coronary circulation. We measured tissue levels of renin (R), angiotensinogen (Aogen), Ang I and Ang II in left and right atrium and ventricle of hearts obtained from normal and nephrectomized pigs (body weight 25–30 kg) within 2–3 minutes after death. Cardiac tissue was homogenized, and both cytosol and membrane fractions were prepared. Measurements of renin were made in the presence and absence of the specific renin inhibitor Ro 42,5892 in order to correct for Ang I generating activity not due to renin. Ang I and Ang II were measured in cardiac homogenates after SepPak extraction and HPLC separation. Cardiac renin (range 1.4–12 pmol Ang I/g per hour), Aogen (range 27.4–125 pmol/g), Ang I (range 5.7–48 fmol/g), and Ang II (range 8.7–193 fmol/g) levels in normal pigs were all considerably higher (2–10 fold) than could be explained by contamination with plasma. The cardiac Ang II/Ang I ratio was 2–3 times higher than in plasma. Cardiac R, Ang I and Ang II were positively correlated and there was a direct correlation between cardiac and plasma R levels. At least 15–20% of cardiac R was membrane-bound and could only be solubilized by the addition of 1% Triton X-100. Renin, Ang I and Ang II, but not Aogen, were undetectable in both plasma and cardiac tissue obtained from nephrectomized animals 30 hours post-nephrectomy. These results support existing evidence for the presence of angiotensin production in cardiac tissue. Renin of renal origin is the most important, if not only, Ang I generating enzyme in the heart. Part of this renin seems to be membrane-bound.

Decreased production of heparan sulfate proteoglycan by cultured human glomerular visceral epithelial cells and human mesangial cells under hyperglycemic conditions. *N.F. van Det, N.A.M. Verhagen, J.T. Tamsma, J. van der Born, J.H.M. Berden, M.R. Daha, J.A. Bruijn, and F.J. van der Woude, Depts. of Endocrinology, Pathology and Nephrology, University Hospitals Nijmegen and Leiden, Nijmegen and Leiden, The Netherlands.* Recently, we have demonstrated a decrease in heparan sulfate glycosaminoglycan (HS-GAG) staining in the glomerular basement membrane of diabetic kidneys. To study the mechanism underlying this phenomenon we cultured human glomerular visceral epithelial cells (GVEC) and human mesangial cells (MC) under normal and hyperglycemic conditions. ^{35}S sodium sulfate (100 μCi/ml) and ^{3}H glucosamine (25 μCi/ml) were used for proteoglycan labeling. Experiments were performed under normal (5 mM glucose) and hyperglycemic (25 mM glucose) conditions. Proteoglycans were isolated after two DEAE chromatography purification steps. The content of each peak was analyzed by sensitivity to nitrous acid, chondroitinase ABC and AC on G50 Sephadex column fractionation. Heparan sulfate was also detected by a sensitive inhibition ELISA using mouse anti-GBM HS-GAG side chain specific antibodies. The majority of macromolecular ^{35}S sulfate and ^{3}H GAG from the HPLC-DEAE column eluted as two partially separated peaks. Peak I contained HSPG and reacted positive in the HS-GAG ELISA for both AMC and GVEC. Peak II from AMC contained dermatan sulphate proteoglycan (DSPG) and from GVEC a mixture of HSPG and chondroitin sulfate proteoglycan(CSPG). We observed a decrease in ^{3}H HSPG/DSPG ratio when MC and GVEC were grown under hyperglycemic condition. ELISA measurements in culture media revealed that high glucose levels decrease HS production by both GVEC (0.31 μg/ml/10^5 cells vs. 0.21/10^5) and MC (0.433/10^5 vs. 0.244/10^5; $P \leq 0.05$). In conclusion: GVEC and MC produce less HS-GAG under hyperglycemic conditions. This may explain the decreased HS-GAG staining *in vivo* observed in kidneys with diabetic nephropathy. A decrease in glomerular HSPG content could theoretically lead to albuminuria and mesangial cell expansion.

Rapid onset of impaired afferent arteriolar (AA) responsiveness to pressure and angiotensin II (Ang II) in rats with experimental diabetes mellitus. *P.M. ter Wee, H. Forster, and M. Epstein, Department of Nephrology, Free University Hospital, Amsterdam, The Netherlands, and Nephrology Section VA Medical Center and University of Miami, Miami, Florida, USA.* Previous studies from our laboratory have demonstrated impaired AA responsiveness to pressure in rats 6 weeks after induction of diabetes mellitus. How rapidly this impairment in autoregulation ensues is controversial. In order to further characterize the temporal profile of the impairment in AA responsiveness, pressure-induced AA vasoconstriction was examined in isolated perfused hydronephrotic kidneys from 1-week diabetic rats (STZ; 55 mg/kg iv). In 8 rats, diabetes was left untreated (DM). In 9 rats, insulin therapy (NOVO ultralente) was initiated the day after the administration of STZ in order to achieve strict metabolic control (DM+ Ins). Hydronephrosis of the right kidney was induced 4–6 weeks prior to the perfusion study to permit videomicroscopy of renal microvessels. Myogenic responsiveness was assessed by stepwise increments in renal arterial pressure (RAP; 80 to 180 mm Hg). Subsequently, Ang II (3.0×10^{-10} M) was added to the perfusate. Vessel diameters were measured by computer-assisted image processing. As RAP was increased, AA from the normal kidneys constricted markedly (17.1 ± 1% diameter decrement at 180 mm Hg; $N = 35$; $P < 0.001$ vs. 80 mm Hg), whereas AA from DM kidneys manifested attenuated constrictions (8.7 ± 2%; $N = 37$; $P < 0.005$ vs. 80 mm Hg; $P < 0.001$ vs. control). Insulin treatment prevented the impairment in AA responsiveness to pressure (18.1 ± 1.6%; diameter decrement at 180 mm Hg; $N = 34$; $P < 0.001$ vs. 80 mm Hg; $P < 0.001$ vs. DM). Ang II-induced AA constrictor responses of DM kidneys was reduced as compared to control rats (23.1 ± 2.7% vs. 41.0 ± 3.2% diameter decrement, respectively; $P < 0.005$). DM + Ins AA manifested constriction of 33.4 ± 2.6% to Ang II ($P < 0.01$ vs. DM; NS vs. control). We conclude that an impairment of AA responsiveness to pressure and Ang II occurs early in the course of experimental diabetes mellitus which can be prevented by insulin treatment.

Effect of atrial natriuretic peptide on renal and vascular permeability in diabetes mellitus. *R. Zietse, F.H. Derkx, W. Weimar, and M.A. Schalekamp, Dept. of Internal Medicine I, University Hospital Dijkzigt, Rotterdam, The Netherlands.* Synthetic human ANP (102–126) 0.01 μg/kg/min or vehicle was intravenously infused for 2 hours in 10 patients with insulin dependent diabetes mellitus and microalbuminuria (albumin excretion 20–200 μg/min) and in 10 control subjects. In the diabetic group, the IgG clearance was higher but both size- and charge-index as calculated from albumin and immunoglobulin clearances were equal compared to controls. The fractional clearances of small dextrans (<36 Å) were lower in diabetics, which was compatible with a depressed hydraulic permeability (K_f). During ANP infusion the excretion of albumin and IgG increased in the diabetic subjects (189 ± 12 to 521 ± 84 μg/min, and 7.1 ± 3.5 to 21 ± 8.1 μg/min, respectively; both $P < 0.05$) but not in the controls. In the diabetics the clearance of dextrans > 54 Å increased and our calculations indicated an increase in "shunt-flow" (ω_0). The transcapillary escape rate of albumin, which was elevated in the diabetics at baseline, increased in the diabetic group only. Thus, ANP uncovers altered size-selectivity of the filtration barrier in a phase that is otherwise characterized by charge-selective changes only. Moreover, the increased susceptibility of the glomerular capillaries in diabetics to ANP seems to be part of a more generalized capillary abnormality, because ANP also increases the transcapillary escape of albumin.

Indirect measurement of lymphatic absorption using intraperitoneally administered inulin. *T. Rowshani, D.G. Struijk, G.C.M. Koomen, A.L.T.*

Imholz, and R.T. Krediet, Academic Medical Center, Amsterdam, The Netherlands. The disappearance rate of intraperitoneally (i.p.) administered macromolecules can be used as an indirect measurement of the fluid loss from the peritoneal cavity by lymphatic absorption (LA) in CAPD patients. The clearance (C) of i.p. solutes out of the peritoneal cavity is 1–2 ml/min larger than the appearance rate in the dialysate after intravenous administration, irrespective of solute size. This difference can be used to estimate LA rate (LAR). As a power relationship is present between the mass transfer area coefficient (MTC) of small solutes or C of macromolecules and their free diffusion coefficient in water, the theoretical C-inulin from the circulation to the dialysate can be interpolated from the MTC-creatinine and C-$\beta_2$microglobulin using the free diffusion coefficient of inulin (theoretical C-inulin). The difference between C-inulin after i.p. administration (C-inulin observed) and theoretical C-inulin (inulin difference), can be used to represent LAR. Fluid and solute kinetics were examined 42 times in 34 stable CAPD patients using 4-hour dwell, dialysate glucose 1.36% to which dextran 70 1 g/liter and inulin 2.5 g/liter were added. Mean duration of CAPD treatment was 28 months. LAR was calculated as the dextran disappearance rate (DDR; standard method) and also as the inulin difference (ΔC-inulin). DDR was 1.22 ± 0.08 ml/min (mean ± SEM) and the ΔC-inulin was 1.66 ± 0.09 ml/min. A good correlation was found between DDR and the ΔC-inulin: $r = 0.60$, $P < 0.001$. Both DDR and the ΔC-inulin were related to net ultrafiltration: DDR: $r = -0.63$, $P < 0.001$; ΔC-inulin: $r = -0.57$, $P < 0.001$. It can be concluded that the ΔC-inulin can be used to estimate LAR in CAPD patients. It also gives additional evidence that DDR is not caused by local accumulation within the peritoneal cavity. LA proved to be an important determinant of net ultrafiltration, irrespective whether DDR or ΔC-inulin was used.

Value of the low-dose desferrioxamine (DFO) test in the diagnosis of aluminum-related bone disease (ARBD) in dialysis (D) patients. *P.C. D'Haese, M.M. Couttenye, L.V. Lamberts, W.G. Goodman, G.D. Nuyts, E. Lemoniatou, P. Digenis, I. Sotornik, A. Fagalde, and M.E. De Broe, Depts. of Nephrology, University of Antwerp, Belgium, UCLA, Los Angeles, California, USA, Athens, Greece, Prague, Czech Republic, and Cordoba, Argentina.* The use of low DFO doses (5 mg/kg) provides an effective and safe treatment of Al overload, raising the question of their diagnostic value as well. In 73 D patients, histological and histochemical (Aluminon^R stain) data and Al bulk analysis of bone biopsies were correlated with the results of a 5 mg/kg DFO test. ARBD was defined as follows: positive Aluminon^R stain (> 0%) with or without increased bone Al (> 15 μg/g) combined with histological features of either adynamic bone disease, mixed disease, osteomalacia and normal histology with a bone formation rate < 250 $\mu m^2/mm^2$/day. The test was considered positive when the post-DFO serum Al increment was above 50 μg/liter and iPTH < 350 ng/liter. Following the above criteria, 31 out of the 73 D patients presented with ARBD. The sensitivity (Se), specificity (Sp) and positive predictive value (PPV) of the 5 mg/kg DFO test were 78, 88, and 83%, respectively. By increasing the iPTH threshold value up to 650 ng/liter, the Se increased up to 94% whereas the Sp decreased to 76%. We conclude that the low DFO test in combination with iPTH determination is a reliable test in the detection of ARBD and accurately predicts the absence of the disease.

A frameshift mutation in the type IV collagen α3 gene in autosomal recessive Alport syndrome. *H. Lemmink, L. van den Heuvel, L. Kluijtmans, T. Mochizuki, S. Reeders, L. Monnens, C. Schröder, H. Brunner, and H. Smeets, Departments of Human Genetics and Pediatrics, University Hospital, Nijmegen, The Netherlands; Boyer Center for Molecular Medicine, Yale University School of Medicine, New Haven, Connecticut, USA.* Alport syndrome is a progressive hereditary glomerulonephritis with a heterogeneous clinical picture. In the majority of families the disease segregates with markers from the Xq22 region, and in 9 of our group of 45 unrelated patients, mutations were identified in the X-linked type IV collagen α5 gene (COL4A5). As yet, 30% of the coding sequence has been analyzed. Still, a number of families exists with no apparent X-linked inheritance, and autosomal dominant or recessive inheritance should be considered. In a Dutch family with two affected siblings, a boy and a girl, results of an intragenic CA-repeat marker excluded the involvement of COL4A5. The girl had severe symptoms of Alport syndrome (progressive renal failure and hearing loss) and she reached end-stage renal disease at the age of 9 years. A kidney transplantation failed, because the child developed progressive anti-glomerular basement membrane (anti-GBM) nephritis against the allograft 6 months after transplantation. Her younger brother suffered from hematuria and hearing loss as well, but his kidneys still functioned normally at the age of 13 years. Their parents were unaffected. Segregation of an intragenic CA-repeat marker was consistent with a possible involvement of the COL4A3 gene, which is located on chromosome 2. Mutation analysis revealed that both patients were homozygous for a 5 bp deletion in the fifth exon of the COL4A3 gene (counting from the 3′ end). The deletion disrupted the reading frame and introduced a premature stop codon. It should produce a truncated protein without a functional NC-domain. A competitive ELISA using the anti-GBM serum confirmed the involvement of the COL4A3 NC domain. In this case the genetic defect explained the target of the antiserum.

Familial glomerulonephritis characterized by massive deposits of fibronectin: An uncommon cause of nephrotic syndrome. *K.J.M. Assmann, R.A.P. Koene, and J.F.M. Wetzels, Depts of Pathology and Nephrology, University Hospital Nijmegen, Nijmegen, The Netherlands.* In 1985 a 40 year old male patient was referred to our clinic because of proteinuria, which was first discovered in 1976, and had increased to nephrotic range over the last year. Serum creatinine was 79 μmol/liter, albumin 27 g/liter and urinary protein excretion averaged 6.4 g/24 hr. In serum no cryoglobulins or paraproteins were found. Light microscopy (LM) of a renal biopsy showed enlarged glomeruli with massive deposits of a homogeneous, eosinophilic material in the subendothelial areas and in the mesangium, in which an increased number of mesangial cells were dispersed. The GBM of the glomerular capillaries that were displaced to the periphery was normal. Staining for amyloid was negative. The deposits only stained faintly for immunoglobulins (heavy and light chains), complement factors C1q and C3, and the extracellular matrix proteins collagen IV and laminin in the immunofluorescence (IF). However, they strongly stained with mAb against fibronectin. By electron microscopy, massive deposits with a fine granular appearance were seen in the mesangium and frequently in the subendothelial spaces. By higher magnification these deposits appeared to be composed of irregularly arranged fibrils of 10–12 μm in diameter, suggestive for fibronectin. Surprisingly, no fusion of epithelial foot processes or influx of inflammatory cells were seen. Nephrotic range proteinuria persisted over the next eight years; however, renal function remained stable. A second biopsy taken eight years later demonstrated similar lesions, with hardly any progression by LM. Recently, a 21 year old son also underwent a kidney biopsy because of a moderate proteinuria. LM showed identical lesions to the biopsy of his father. Using mAb specific for cell-derived fibronectin (IST-9) and plasma and cell-derived fibronectin (IST-4), we could additionally demonstrate that the fibronectin deposited in the glomeruli was derived from the plasma. In conclusion, we present a rare form of familial glomerulonephritis (GN) that is characterized by massive deposits of fibrillar arranged fibronectin without any inflammatory response. This GN has an indolent course with hardly any deterioration of renal function.

Rapid inactivation of C5b-9 by vitronectin and CD59, but not CD55 determines the rat strain-dependent development of proteinuria in anti-Thy-1 nephritis. *D. Salazar, M.R. Daha, A.v.d. Wal, J.A. Bruijn, Ph.J. Hoedemaeker, W.G. Couser, and E. De Heer, Depts. of Pathology and Nephrology, University of Leiden, Leiden, The Netherlands, and University of Washington, Seattle, Washington, USA.* Injection of rats with anti-Thy-1 antibodies results in the rapid development of proteinuria in Wistar, BN, AO and F344 rats (max. at day 3), but no proteinuria in PVG and MAXX rats. Genetic studies with MHC-congenic strains revealed that the observed strain differences were governed by non-MHC genes. We then investigated whether differences in the possible inactivation of C5b-9 complexes by complement inhibitors were responsible for these differences in proteinuria development. Irrespective of the development of proteinuria in all rat strains, we observed an increasing mesangial expression of CD55 (DAF) within 24 hours after injection of ER4. Double label IF with OX7 (IgG1 anti-Thy-1) demonstrated an exclusive expression of CD55 on mesangial cells. Mesangial CD55 expression was shown to colocalize with C5b-9 complexes as detected by a monoclonal antibody against rat C5b-9 neoantigen. In non-proteinuric strains C5b-9 complexes persisted in the mesangial area for at least 7 days and they immediately (within 1 hr) colocalized with vitronectin and CD59 (HRF-20, 4 hrs). However, in proteinuric rats C5b-9 complexes were removed within 6 days from the mesangial area. In these rats mesangial colocalization with either vitronectin, or CD59, could not be observed. Pretreatment of PVG rats

with antibodies against either vitronectin or CD59, followed by administration of anti-Thy-1 antibodies, resulted in immediate development of proteinuria. This study shows that the susceptibility of complement-mediated damage in the glomerulus may be related to genetic differences in its capability to inactivate Cb5-9 complexes by complement inhibitors.

Albuminuria in the heterologous phase of anti-GBM nephritis in beige mice is not mediated by platelets or fibrin. *G.W. Feith, K.J.M. Assmann, M.J.J.T. Bogman, A.P.M. van Gompel, J. Schalkwijk, and R.A.P. Koene, Depts. of Pathology, Dermatology, and Nephrology, University Hospital Nijmegen, Nijmegen, The Netherlands.* Beige mice, which are deficient for leukocytic neutral proteinases, do not develop albuminuria within 24 hours after injection of rabbit anti-mouse GBM Ig (RaMGBM). After 24 hours, however, a gradually increasing albuminuria is observed which is maximal at day 5. Albuminuria is preceded by an influx of PMN in the glomeruli and progressive intravascular coagulation. Therefore, we have now studied the role of platelets and fibrin in the development of the albuminuria in the late heterologous phase of passive anti-GBM nephritis in beige mice. Platelet depletion was induced and maintained by repeated i.p. administration of rabbit anti-mouse platelet Ig (mean platelet count throughout the entire experimental period 17×10^9/liter vs. 395×10^9/liter, $P < 0.002$). Controls were treated with normal rabbit Ig. PMN influx in the glomeruli 2 hours after the induction of the nephritis was comparable in both groups (4.1 ± 0.4 PMN/glomerular cross section vs. 5.2 ± 1.3 in the control group). On day 3 after the i.v. injection of RaMGBM, neither albuminuria [10301 ± 6067 μg/18 hr ($N = 8$) in the experimental group vs. 7835 ± 6320 μg/18 hr ($N = 11$) in controls] nor the extent of the glomerular damage as assessed by light microscopy, were influenced by the thrombocytopenia. By immunofluorescence (IF), fibrin deposits were present in comparable amounts in both groups. Fibrinogen depletion by the administration of Ancrod (blood fibrinogen <120 mg/liter in Ancrod treated mice vs. 2131 ± 601 mg/liter in untreated controls) also did not alter the extent of the albuminuria (12256 ± 9697 μg/18 hr vs. 7443 ± 5365 μg/18 hr) nor the histological changes. In both groups fibrin deposits could be detected in comparable amounts by IF. Ultrastructurally, however, fibrin was not deposited as polymerized fibrin in the Ancrod treated group. We conclude that neither platelets nor fibrin are important mediators of the glomerular damage and the albuminuria occurring in the late heterologous phase after the injection of RaMGBM in beige mice.

Molecular construction of glomerular sclerotic lesions in experimental immune complex nephritis. *E.C. Bergijk, J.J. Baelde, E. de Heer, P.D. Killen, and J.A. Bruijn, Depts. of Pathology, University of Leiden, Leiden, The Netherlands, and University of Michigan, Ann Arbor, Michigan, USA.* Induction of chronic serum sickness by repetitive injections of human IgG into preimmunized Wistar rats leads to the development of immune complex nephritis of which glomerulosclerosis is a severe complication. The aim of this study was to elucidate the role of the extracellular matrix (ECM) in the development of glomerulosclerosis in this model. Proteinuria increased when injection of human IgG had been started. Injections were stopped when the rats had developed proteinuria exceeding 800 mg/24 hr. This moment was considered week 0. At this early stage fibrinogen accumulation was observed along the endothelial cells, probably related to damage of the endothelial lining. mRNA levels for several collagen types, laminin B1 and B2, and fibronectin (fn) were significantly increased in both whole-kidney tissue and in isolated glomeruli, but morphological changes were not observed. *In situ* hybridization experiments demonstrated increased ECM mRNA levels in glomerular and tubular cells. Starting at week 15, glomerular mesangial matrix expansion and thickening of the glomerular basement membrane (GBM) were observed. Several ECM components, including laminin, fn, and collagen types IV, and VI, were abundantly present. Coagulation factors were not observed at this moment. ECM mRNA levels were decreased as compared to week 0, but were still above normal. Focal and segmental end-stage sclerotic lesions developed at weeks 25–30. Fn and fibrinogen were the major constituents of these lesions. Other ECM components were found peripherally from these lesions in the remnants of the mesangial matrix and GBM. Sclerotic matrices did not demonstrate an increase of cellular-fn, and other constituents from the circulation were not present in the lesions. Glomerular ECM mRNA was decreased to normal levels. However, a dramatic increase of fn and α1(IV)col mRNA expression was observed at sites of inflammatory infiltrate in the perivascular, interstitial, and periglomerular regions. These facts, together with accumulation studies, strongly suggest that in the final stage of chronic serum sickness there is a specific accumulation of exogenous fn in the end-stage sclerotic lesions in glomeruli, and that simultaneously an interstitial inflammatory reaction takes place leading to increased ECM production in the tissue surrounding the damaged glomeruli.

Changes in the glomerular filtration barrier induced by contact between a human plasma factor and kidney tissue. *P.K. Cheung, A. Boes, W.W. Bakker, Department of Pathology, University of Groningen, Groningen, The Netherlands.* The pathogenesis of minimal change nephrotic syndrome (MCNS) is largely unknown. Several circulating factors have been suspected to play a role in the pathogenesis of MCNS; however, a causal relationship between the activity of such factors and the glomerular lesion is lacking. Previously, we have described a heat labile vasoactive factor in normal human plasma (100 kF, molecular wt ± 100 kD) which is able to induce glomerular alterations in the rat kidney characteristic for MCNS, such as diminished subendothelial heparan sulphate proteoglycans, anionic sites, and reduction of glomerular sialoglycoproteins (GSP). In addition it was shown that in plasma of subjects with MCNS during relapse, 100kF in activated form was detected. Since we recently also observed reduced stainability for glomerular ectonucleotidase (ATP/ADPase) in biopsies from subjects with MCNS (in contrast to other forms of glomerulopathy), the question emerged whether 100kF is able to induce this effect. Therefore, partially purified 100kF solutions [6.0 mg protein in 4.0 ml phosphate buffered saline (PBS) pH 7.4 at 37°C] was perfused for 6 minutes into the left kidneys of anesthetized Wistar rats ($N = 6$) *ex vivo* according to standard methods. Contralateral kidneys were treated identically with heat-inactivated (HI) 100kF. Cryostate sections of both kidneys were stained for GSP using colloidal iron, and for ATP/ADPase activity using standard enzyme histochemistry. These stainings were also done using: (a) rat ($N = 8$) and human kidney sections ($N = 5$), and (b) confluent human endothelial cell cultures ($N = 6$), following *in vitro* incubation with either 100kF in various concentrations (0.5; 1.0; 1.5; 2.0 and 2.5 mg/ml PBS) or HI material for 2.0 hours at 37°C. The results show a significant reduction of stainability for GSP and glomerular ATP/ADPase after 100kF perfusion, while *in vitro* incubation with 100kF of rat and human kidney sections showed similar results in a dose-dependent manner. Also, in human endothelial cell cultures a significant dose dependent decrease of stainability for GSP and ectonucleotidase could be detected following 100kF incubation. It is concluded that 100kF is able to affect glomerular and endothelial GSP as well as ectonucleotidase. Since biopsies from subjects with MCNS show decreased GSP and a diminished number of subendothelial anionic sites, whereas ectonucleotidase (in the rat kidney predominantly located along the subendothelial part of the GBM) is also injured by this factor, we feel that 100kF activated *in vivo* may affect the endothelial and subendothelial part of the glomerular filtration barrier in subjects with MCNS.

Apoptosis during and after regeneration from gentamicin toxicity in the rat kidney. *E.J. Nouwen, M.Q. Zhu, and M.E. De Broe, Dept. of Nephrology, University of Antwerp, Antwerp, Belgium.* Female Wister rats (200–250 g) were s.c. injected 3 times daily with gentamicin (400 mg/kg/day) during 2 days and were sacrificed at regular time intervals over a period of 10 days. During this period acute tubular necrosis confined to the proximal tubules, followed by regeneration, could be observed. The occurrence of apoptosis or programmed cell death was quantitated in cortical proximal and distal tubules during injury and regeneration. Apoptosis, which was absent in controls, was rare in the injured and regenerating PCT before day 10: approximately one apoptotic cell was seen in every 10 tubular cross-sections. However, during the development of severe proximal tubular necrosis, apoptosis started to occur in cortical distal tubules at day 1, reached a peak at day 2, and gradually declined thereafter. It parallelled the increased proliferative activity in the distal tubules in this period. Upon completion of morphological recovery of the PCT at day 10, this segment had become hyperplastic, and a 5-fold increase in apoptosis was observed. In conclusion: (1) Apoptosis in morphologically undamaged distal tubules could be the consequence of tubular obstruction during the first days after induction of acute renal failure; (2) The enhanced occurrence of apoptosis in the PCT early after completion of regeneration may provide a mechanism to normalize the excess in cell number.

Differential binding of PR3 and MPO to monolayers of HUVEC. ANCA mediated ADCC of PR3/MPO incubated HUVEC? *B.E.P.B. Ballieux, K.*

Zondervan, P. Kievit, E.C. Hagen, F.J. van der Woude, L.A. van Es, and M.R. Daha, Dept. of Nephrology, University Hospital Leiden, Leiden, The Netherlands. **Introduction:** Binding of both PR3 and MPO to endothelial cells (EC) has been suggested to be involved in the vascular damage seen in patients with Wegener's Granulomatosis or microscopic poliangiitis. ADCC and complement or neutrophil mediated mechanisms in the presence of anti-neutrophil cytoplasmic antibodies (ANCA) have been proposed. We investigated in detail the interaction of MPO and PR3 with cultured human EC and its matrix products. We also performed ADCC experiments on PR3/MPO incubated HUVEC monolayers after interaction with ANCA positive sera or rabbit anti-PR3/MPO. **Methods:** ELISA's were performed with PR3 coated onto monolayers of HUVEC and rabbit IgG directed against PR3. MPO was coated under similar conditions onto monolayers of HUVEC and directly detected using a peroxidase substrate (ABTS). Before adding the substrate in some experiments the endothelial cells were detached from the extracellular matrix with PBS/EDTA and the substrate was added separately to the cells and to the remaining matrix. For ADCC, ^{51}Cr labeled monolayers of HUVEC were coated with PR3/MPO, incubated with anti-PR3/MPO and incubated for 6 hours with PBMC. **Results:** Both MPO and PR3 strongly bind to cultures of HUVEC. Separation of the endothelial cells and the extracellular matrix before adding the substrate showed binding of MPO both to the cells and to the matrix. PR3 only bound to the matrix. Bound MPO could not be detected by anti-MPO. No ADCC reactivity mediated by anti-PR3/MPO was found against PR3/MPO coated HUVEC monolayers. **Discussion:** The results described above show differences in the binding properties of MPO and PR3 to EC. In contrast to MPO, PR3 binds mainly to the extracellular matrix, possibly explaining the lack of anti-PR3 mediated ADCC. MPO is probably bound to cryptic sites out of reach for anti-MPO antibodies.

Differential vascular endothelial expression of adhesion molecules in a rabbit model of granulomatous vasculitis. *J.W. Cohen Tervaert, M.I. Cybulsky, M.A. Gimbrone, Jr., Vascular Research Division, Dept. of Pathology, Brigham and Women's Hospital, Boston, Massachusetts, USA.* Granulomatous vasculitis, characterized by angiocentric mononuclear leukocyte accumulation, can be induced by intravenous injection of β-glucan particles (S. cerevisiae) in rabbits, as previously described in rats. In this study, we studied endothelial-dependent mechanisms during development of pulmonary lesions. New Zealand white rabbits (1.5–2 kg) were injected with 15 mg/kg β-glucan and sacrificed at different time intervals. Immunohistochemical staining was performed on frozen sections with Mab Rb1/9 (VCAM-1), 14G2 (E-selectin), and Rb 2/3 (ICAM-1). Northern blot analysis of RNA extracted from lungs was done with rabbit VCAM-1, ICAM-1, E-selectin, interleukin-8 (IL-8), and monocyte chemoattractant protein-1 (MCP-1) cDNA probes. Immediately after glucan injection, embolization with glucan aggregates was found in small pulmonary vessels. At 4 hours, a localized, inflammatory response around glucan aggregates in the lumen of these vessels was found. VCAM-1 and ICAM-1 staining was diffusely and markedly increased in vessels with and without emboli, whereas E-selectin was only focally and minimally expressed in a few vessels. At 24 hours, mural vasculitis with destruction of vessel walls was detected. Many glucan particles were at least partly extravascularly localized, surrounded by monocytes and macrophages. Endothelial expression of VCAM-1 and ICAM-1 was markedly increased, whereas E-selectin was absent. At 4–5 days, there were well-demarcated interstitial granulomas and virtually all glucan particles were now found extravascularly, engulfed by macrophages. Endothelial ICAM-1 and VCAM-1 staining was reduced to nearly normal levels and E-selectin was absent. At 4 hours, a clear increase in VCAM-1, ICAM-1, IL-8 and MCP-1 mRNA expression was observed, whereas no increase in E-selectin mRNA expression was found. At 24 hours, mRNA for IL-8 was still increased, whereas mRNA levels of VCAM-1, ICAM-1, E-selectin, and MCP-1 were comparable to those found in control rabbits. This model may be useful for the further analysis of molecular regulatory mechanisms responsible for the differential regulation of endothelial-dependent processes in an integrative pathophysiological setting.

Hypertriglyceridemia in nephrotic syndrome is only partially due to increased hepatic fatty acid (FA) synthesis and triglyceride secretion. *J.A. Joles, C. Bijleveld, A. van Tol, M.J.H. Geelen, and H.A. Koomans, Nephrology, Medical Faculty and Biochemistry, Veterans Faculty, Utrecht; Biochemistry, Erasmus University, Rotterdam, The Netherlands.* Hepatic cholesterol synthesis and secretion are stimulated by low plasma oncotic pressure (π) in nephrotic syndrome (NS) causing hypercholesterolemia. On the other hand, hypertriglyceridemia has been associated with delayed catabolism of triglyceride (Tg)-rich particles. It is unknown whether hepatic FA synthesis and Tg secretion are also stimulated by decreased π. Thus, we measured activity of 2 key enzymes of FA synthesis in hepatic cytosol: acetyl CoA carboxylase (ACC) and FA synthase (FAS) from control (CON) male Sprague-Dawley (SD) rats, and rats with either congenital analbuminemia (NAR) or SD rats with adriamycin-induced NS. In a second cohort we measured post-Triton Tg secretion rates (TgSR; $nmol \cdot min^{-1}$), assuming a plasma volume of 4% body weight (body wt).

	CON	NAR	NS
N	10	7	9
Proteinuria $mg \cdot d^{-1}$	30 ± 4	14 ± 2	811 ± 45[ab]
Plasma π *mm Hg*	18.3 ± 0.7	13.2 ± 0.3[a]	10.7 ± 0.4[ab]
Plasma apo B *AU*	88 ± 10	168 ± 8[a]	285 ± 35[ab]
ACC/g body wt	100 ± 6	145 ± 11[a]	139 ± 15[a]
% of CON			
FAS/g body wt	100 ± 5	165 ± 19[a]	215 ± 31[a]
% of CON			
N	10	6	9
Plasma Tg $mmol \cdot l^{-1}$	0.51 ± 0.05	2.13 ± 0.16[a]	3.38 ± 0.47[ab]
TgSR $nmol \cdot min^{-1}$	0.52 ± 0.03	0.69 ± 0.06[a]	0.69 ± 0.05[a]

[a] $P < 0.05$ vs. CON; [b] $P < 0.05$ vs. NAR; Abbreviation AU is arbitrary units.

In conclusion, hepatic FA synthesis capacity, monitored by ACC and FAS, as well as TgSR, were increased in rats with low π. In addition, however, neither FA synthesis nor TgSR was higher in NS rats than in NAR, although π was lower in NS rats than in NAR. Therefore, higher Tg levels in NS rats, as compared to NAR, are probably due to a proteinuria-related disturbance in Tg catabolism.

Renin-inhibition restores pressure natriuresis (PN) in essential hypertension (EH). *P. van Paassen, G.J. Navis, P.E. de Jong, and D. de Zeeuw, University Hospital, Groningen, The Netherlands.* One of the features of EH is an abnormal PN. Experimental evidence suggests a mechanistic role for the renin-angiotensin system (RAS). We tested this hypothesis by measuring PN relations and PRA in 7 hospitalized ambulant EH patients both before and after one week treatment with the specific renin-inhibitor Remikiren (Rem, 600 mg oral compound, at noon). Patients were on a diet containing 50 mmol Na, 100 mmol K and 2500 ml fluid. PN was evaluated by correlating the daily fluctuations in ambulatory blood pressure (every 15 minutes, Spacelabs) and U_{Na} (every two hours), both over a period of 48 hours. Before Rem, mean arterial pressure (MAP) was 109.4 ± 1.2 mm Hg and 94.8 ± 1.3 during day and night, respectively; UNa was 38.5 ± 3.2 mmol/24 hrs and PRA (12.00 hr) was 2.7 ± 0.4 nmol/liter/hr. During Rem, an accumulated Na loss of 68 (−25 to 200) mmol occurred. When sodium excretion had stabilized at 39.2 ± 3.1 mmol/24 hrs, MAP was 100.1 ± 1.2 and 89.7 ± 1.3 mm Hg during day and night, respectively, and PRA was 1.1 ± 0.1 nmol/liter/hr at trough. The mean changes in MAP and UNa did not correlate ($r = 0.31$, NS) before Rem, whereas the PN relation was restored after Rem treatment ($r = 0.66$, $P < 0.01$). Indeed, individual patients showing a poor PN relation showed a restoration of PN upon treatment, whereas the PN relation improved in those with an already significant PN relation before treatment ($P < 0.02$). Interestingly, the presence or absence of a significant PN relation before treatment was explained by the initial PRA (higher PRA values corresponded with poorer PN relation; $r = 0.78$, $P < 0.05$). Moreover, the treatment induced change in PN correlated with the decrease in PRA ($r = 0.81$, $P < 0.05$). In conclusion, the RAS plays an important role in the PN defect, since interference in the RAS with a specific renin-inhibitor restores this defect.

The acute effects of ACE-inhibition (ACEi) on hippuran (Hip) handling in normal and adriamycin (ADR) nephrotic rats. *W.D. Kloppenburg, G.J. Navis, P.E. de Jong, and D. de Zeeuw, State University Hospital, Groningen, The Netherlands.* Kidney diseases and vasoactive drugs can reduce the renal extraction of Hip (EHip), affecting the reliability of this tracer to estimate renal plasma flow (RPF). The effect of ADR nephropathy on EHip and the acute effect of ACEi on Hip handling in nephrosis has never

been investigated. We, therefore, studied EHip in normal (C, $N = 11$) and nephrotic male Wistar rats, on a low sodium intake, before and after ACEi. Nephrosis was induced by a single injection of ADR (2.5 mg/kg), resulting in a stable proteinuria of 466 ± 62 and 659 ± 68 mg/24 hr at 4 weeks (A1, $N = 10$) and 12 weeks (A2, $N = 8$), respectively. Clearances and extraction ratios of the tracers ^{131}I-Hip and ^{125}I-Iothalamate, mean arterial pressure (MAP) and urine volume (UV) were measured before and after lisinopril (10 mg/kg i.v.) under halothane anesthesia. Baseline measurements and changes after ACEi are listed in the table.

	EHip	ERPF	RPF	GFR	MAP	UV
	E ratio %	*ml/min/100 g body wt*			*mm Hg*	*µl/30 min*
C	0.75 ± 0.02	2.49 ± 0.12	3.35 ± 0.21	0.75 ± 0.04	93 ± 3	360 ± 19
A1	0.72 ± 0.01[a]	2.20 ± 0.09	3.10 ± 0.15	0.65 ± 0.03[a]	97 ± 2	397 ± 26
A2	0.73 ± 0.01	1.88 ± 0.14[a]	2.59 ± 0.17[a]	0.53 ± 0.05[a]	102 ± 3[a]	417 ± 49
		% change from baseline after ACEi				
C	−19 ± 5[b]	−9 ± 5	15 ± 6[b]	−22 ± 4[b]	−34 ± 4[b]	−38 ± 11[b]
A1	−11 ± 3[ab]	19 ± 4[ab]	35 ± 5[ab]	−1 ± 4[a]	−20 ± 3[ab]	12 ± 13[a]
A2	−2 ± 2[a]	17 ± 3[ab]	21 ± 5[b]	3 ± 4[a]	−18 ± 3[ab]	22 ± 9[a]

Data are mean ± SEM. [a] $P < 0.05$ vs. C; [b] $P < 0.05$ vs. baseline. Wilcoxon 2-sided analysis.

The EHip, although numerically lower, was well preserved in ADR rats, despite renal function impairment. ACEi induced a fall in EHip in normal and ADR rats. The changes varied considerably between the rats and were more pronounced in normal rats. Combining all groups, the fall in EHip correlated with the drop in MAP ($r = 0.48$, $P < 0.001$), UV ($r = 0.61$, $P < 0.01$), and GFR ($r = 0.56$, $P < 0.01$). In conclusion, in ADR rats the reliability of clearance measurements to estimate RPF is similar as in normal rats. After acute ACEi no reliable RPF estimation can be made, due to a fall in EHip. This fall in EHip was especially prominent when pronounced decreases in GFR and UV occurred, probably due to a decreased filtration pressure associated with a sharply decreased MAP in these rats. The attenuated effects of ACEi in the nephrotic rats compared to the normal rats suggest that the renin angiotensin system may be suppressed in ADR nephrosis.

Assessment of renal viability before transplantation by ^{31}P magnetic resonance. *R.J. Hené, J. van der Grond, W.P.Th.M. Mali, and H.A. Koomans, Depts. of Nephrology and Radiodiagnosis, Utrecht University Hospital, Utrecht, The Netherlands.* In 30% of all transplantations the kidney shows a delayed function, whereas in about 5% the transplants will never start functioning, mostly due to bad conditions before or during preservation. These numbers will increase when kidneys of non-heart beating donors (NHB) are being used. A non-invasive method to predict renal viability before transplantation would therefore be very helpful. We investigated the efficacy of ^{31}P magnetic resonance spectroscopy (MRS), a non-invasive technique of mapping phosphorus metabolism of intact biological systems, performed during the preservation period, to predict postoperative graft function. We performed ^{31}P MRS on 42 human transplant kidneys preserved in HTK solution (a preservation solution not containing phosphate). Kidneys were derived from 33 donors: 5 living related, 22 heart beating, and 6 NHB. The ratio of the phosphomonoester peak (Pm), mainly composed of AMP, and the inorganic phosphate peak (Pi) was used as a parameter for viability. In kidneys showing immediate function after transplantation we found a relation between the Pm/Pi ratio and the time between nephrectomy and MRS ($r = -0.74$, $P < 0.0001$, slope −0.036). Using this equation the Pm/Pi value at nephrectomy and at implantation could be extrapolated. Kidneys from non-heart beating donors had, at nephrectomy, lower estimated Pm/Pi ratios than those of heart beating donors (2.14 ± 0.29 and 1.35 ± 0.40, respectively). In the NHB group and heart beating derived kidneys Pm/Pi ratios were lower in those which showed delayed graft function ($P < 0.05$), when extrapolated for nephrectomy as well implantation time. When 2 kidneys from the same donor were measured, generally there was no difference in Pm/Pi ratio. In 4 cases, however, 1 kidney had a lower ratio than the other. In these cases a delayed graft function occurred only in the kidneys with the lowest ratio. This might suggest that preservation quality might differ even within kidneys from the same donor. In conclusion, we showed that 31P MRS may predict postoperative graft function. The sensitivity to predict delayed graft function was 73%, specificity 84%, and efficacy 81%.

Simultaneous reduction of blood pressure and proteinuria by chronic angiotensin converting enzyme (ACE)-inhibition in hypertensive fawn-hooded (FHH) rats. *A.P. Provoost, L.T. Sterk, G.H. Verseput, and J.J. Weening, Erasmus University, Rotterdam, and AMC, Amsterdam, The Netherlands.* The FHH rat is a model for spontaneous focal glomerulosclerosis (FGS), characterized by early systemic and glomerular hypertension and proteinuria (UpV). ACE-inhibition has been shown to be very effective in the prevention of renal damage in various models of FGS. However, the efficacy to reduce damage in rats with advanced renal disease is not well-known. We tested the effects on SBP, UpV, FGS, and glomerular volume (V_G) of 4 ACE-inhibitors in 25 male, 6 month-old FHH rats with established hypertension and UpV. Dosages per liter drinking fluid were: lisinopril (L), 100 and 25 mg/liter; enalapril (E), 200 and 100 mg/liter; captopril (C), 600 and 200 mg/liter; fosinopril (F), 100 mg/liter. Treatment groups consisted of 3–6 rats. Results were compared with those of 6 control (CON) FHH rats. During 15 weeks of treatment SBP and UpV were determined every 3 weeks. After treatment the incidence of FGS and V_G was assessed.

	SBP_0	SBP_{15}	UpV_0	UpV_{15}	FGS	V_G
	mm Hg		*mg/day*		*%*	*$\mu m^3 \cdot 10^{-6}$*
CON	155 ± 8	162 ± 3	193 ± 22	392 ± 44	28 ± 5	2.54 ± 0.23
L-100	150 ± 2	109 ± 5[a]	212 ± 49	27 ± 7[a]	8 ± 3	1.97 ± 0.11
L-25	150 ± 2	108 ± 3[a]	232 ± 34	67 ± 17[a]	11 ± 1	2.19 ± 0.30
E-200	151 ± 8	108 ± 1[a]	227 ± 37	41 ± 3[a]	5 ± 2	2.57 ± 0.14
E-100	168 ± 3	124 ± 3[a]	226 ± 33	169 ± 32[a]	18 ± 3	2.36 ± 0.25
C-600	153 ± 5	118 ± 1[a]	232 ± 12	77 ± 7[a]	10 ± 6	2.31 ± 0.21
C-200	162 ± 4	139 ± 3[a]	206 ± 20	275 ± 41[a]	25 ± 4	2.25 ± 0.26
F-100	148 ± 2	153 ± 4	234 ± 7	406 ± 52	45 ± 12	2.38 ± 0.11

All data are mean ± SD; [a] $P < 0.05$ vs. CON; ANOVA.

Dose-dependent reductions in SBP and UpV were found for L, E, and C. At the only dose used, F had no effect. Regression analysis showed a close correlation between SBP and UpV during treatment. Furthermore, the rise in UpV between 3 and 15 weeks correlated with the average SBP during that period. At autopsy, the incidence of FGS was correlated with final SBP and UpV. No significant correlation was found between V_G and SBP, UpV, or FGS. In conclusion, in FHH rats with progressive renal damage, chronic ACE-inhibition can effectively reduce SBP, UpV, and the incidence of FGS without affecting in V_G.

Vascular rejection (VR) after kidney transplantation occurs early and is a major determinant of both short-term and long-term survival. *J.L.C.M. van Saase, F.J. van der Woude, J. Thorogood, A.A.M. Hollander, L.A. van Es, and J.A. Bruijn, Department of Nephrology, Leiden University Hospital, and The Eurotransplant Foundation and University Dept. of Medical Statistics, Leiden, The Netherlands.* Vascular rejection is thought to be the major cause of graft loss after the first year after kidney transplantation. To investigate the time of onset of vascular rejection we studied all consecutive patients ($N = 482$) receiving a post-mortal kidney between 1983 and April 1991 in our center. We report findings from biopsies taken during the first three months after transplantation and the consequences for long-term graft survival and risk factors for developing a specific type of rejection. Ninety-three patients lost their grafts due to rejection during a 9-year follow-up period. One year graft survival was 88.9% for 241 patients without rejection, 87.4% for 111 patients with interstitial rejection (IR), and 50% for 84 patients with VR. Five-year graft survival for these groups was 74.4%, 72.1%, and 35.8%, respectively. The relative risk of graft loss was 4.35 (95%, CI 2.89–6.55) for VR and 1.20 (95%, CI 0.75–1.92) for IR compared to patients without rejection during the first 3 months. Risks were calculated with the Cox proportional hazards regression model with VR and IR as time dependent co-variables. Yearly graft loss due to chronic rejection was 2%/year in patients without early rejection, 2.%/year in patients with IR, and 7.7% in patients with VR. Major risk factors for developing vascular rejection were use of azathioprine without cyclosporin, number of HLA-DR mismatches, prolonged cold ischemia time, and previous transplantations. Risk factors for IR

were use of azathioprine without cyclosporin, recipient age and HLA-DR mismatches. We conclude that VR occurs within a short time period following renal transplantation (within 3 months), and is a very strong predictor of graft loss while IR has a minor negative effect on graft survival. HLA-DR mismatching is a strong risk factor for developing rejection.

Conversion from cyclosporin (CsA) to azathioprine (Aza) after cadaveric kidney transplantation (KT): In the long run a better renal function, less hypertension and equal graft survival. *A.A.M.J. Hollander, J.L.C.M. van Saase, A.M.M. Kootte, W.T. van Dorp, H.H. van Bockel, L.A. van Es, and F.J. van der Woude, Dept. of Nephrology and Dept. of Surgery, University Hospital Leiden, Leiden, The Netherlands.* In the period from 1983–1988, 128 patients who received a first or second cadaveric kidney graft participated in 2 prospective, randomized trials in which CsA was either continued or replaced by Aza at 3 months after KT. All patients received high doses of CsA (16 mg/kg/day tapered to 10 mg/kg/day depending on CsA blood levels) and low doses of prednisone (initial dose 20 mg/day tapered to 10 mg/day). Three months after KT patients were randomly assigned to continue CsA (N = 68) or were converted from CsA to Aza (N = 60). In the CsA group the drug dose was tapered to 5 mg/kg/day, depending on CsA blood levels. In the converted group the prednisone was temporarily increased to 25 or 40 mg/day and was tapered off to 10 mg/day. There were no differences between the two groups in age, sex, number of KT, HLA mismatches, panel reactive antibodies, and cold and warm ischemia time. The long-term follow-up (FU) of these patients showed an equal mortality rate in both groups (CsA: 13.2% vs. Aza: 11.7%). Graft survival after 2, 5 and 8 years was 88.4%, 75.4%, and 66.5% for CsA patients and 88.3%, 78.3%, and 76.3% for Aza patients (P = 0.53). Renal function was considerably better in the Aza group: after 1 year the mean creatinine clearance in the converted patients was 78 ml/min versus 60 ml/min in the CsA patients (difference 18 ml/min; 95% CI: 8.1–27.5). This difference remained the same during 8 years of FU. Less proteinuria (> 0.5 g/day) was found in the Aza patients at 5 years (30% vs. 48% in the CsA patients; $P < 0.05$). There was no difference in blood pressure between the 2 groups before KT, at 3 months and during FU, but CsA patients needed significantly more antihypertensive drugs: after 2 years 87% versus 58% of Aza patients. This difference did not change during FU. We conclude that elective conversion at 3 months post-transplant can be done safely and that beneficial long-term effects were found regarding graft survival, renal function, proteinuria, and hypertension.

Modulating side effects of OKT3 by variation of the time point of methylprednisolone (MPNS) administration: Postponement and put off. *F.J. Bemelman, P.Th.A. Schellekens, S. Buysmann, F. van Diepen, S. Surachno, and R.J.M. ten Berge, Renal Transplant Unit and Department of Clinical Immunology, Department of Internal Medicine, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands.* OKT3, a murine IgG2a monoclonal antibody, used in prevention and treatment of allograft rejection, induces severe clinical side effects, which are partly cytokine-mediated. Cytokine release is mediated by OKT3 coated lymphocytes. Steroids induce lymphocytopenia with a nadir 6 hours following administration. To diminish adverse reactions OKT3 is preceded by MPNS. The aim of the study was to determine the optimal time interval between MPNS and OKT3 administration. Three groups of renal transplant patients (pts) treated for acute rejection with 5 mg OKT3 for ten consecutive days were studied: 10 pts received 500 mg MPNS 6 hours before OKT3 (−6 group); 10 pts received 500 mg MPNS 1 hour before OKT3 (−1 group); and 6 pts received 250 mg MPNS 6 and 1 hours before OKT3 (−6/−1 group). We measured: clinical side effects by a score grading from 1 to 6; body temperature; TNF and IL-6 by ELISA. Values are given as mean ± SEM, and a P value of 0.05 was considered significant. **Results:** (1) Side effects score in the −6/−1 group was significantly lower compared to the −1 group and −6 group (1.0 ± 0.25, 2.9 ± 0.48 and 2.3 ± 0.47, respectively; (2) Peak body temperature in the −6/−1 group was significantly decreased compared to the −1 group and −6 group (37.7 ± 0.48°C, 38.8 ± 0.16°C, and 39.4 ± 0.2°C, respectively). Peak temperature in the −6 group was postponed compared to the −1 and −6/−1 group; (3) Peak TNF levels were significantly increased in the −1 group compared to the −6 group and the −6/−1 group (1132 ± 154 pg/ml, 553 ± 138 pg/ml, and 612 ± 128 pg/ml, respectively); (4) Peak IL-6 levels were significantly increased and postponed in the −6 hour group compared to the −1 and −6/−1 group (190 ± 65 pg/ml 6 hrs following OKT3, 89 ± 50 pg/ml and 29.1 ± 16 pg/ml 3 hrs following OKT3, respectively). **Conclusion:** MPNS should be administered 6 hours and 1 hour prior to OKT3.

Additive antiproteinuric effect of ACE inhibition (ACEi) and a low protein diet (LPD) in human renal disease. *R.T. Gansevoort, D. de Zeeuw, and P.E. de Jong, University Hospital, Groningen, The Netherlands.* Both ACEi and LPD are reported to prevent progressive renal function decline. The renoprotective effect of both regimens has been suggested to be partly related to the proteinuria lowering effect. Whether the combination of ACEi and LPD might have an additive value is unknown. We therefore studied the antiproteinuric effect of ACEi, LPD, and their combination in 14 proteinuric patients with stable, non-diabetic renal disease. The protocol consisted of two parallel studies, each of 4 periods. Measurements took place at the end of each of the two months, lasting for the study period. In part I, the additive effect of ACEi to LPD was investigated. In part II, vice versa, the additive effect of LPD to ACEi was investigated. Normal protein diet (NPD) was defined as 1.5 g protein/kg body wt (when C_{Cr} >70 ml/min), or 1 g protein/kg body wt (when C_{Cr} <70 ml/min), while LPD was defined as a 50% decrease in prescribed protein intake during NPD. ACEi was achieved with enalapril 10 mg o.i.d.

Part I	NPD	LPD	LPD + ACEi	LPD
MAP *mm Hg*	95 ± 3	92 ± 3	83 ± 3[ab]	95 ± 5
U_{urea} *mmol/24 hr*	411 ± 59	212 ± 20[a]	230 ± 24[a]	228 ± 25[a]
U_{prot} *g/24 hr*	7.5 ± 0.9	6.2 ± 0.8[a]	3.5 ± 0.7[ab]	5.5 ± 0.9
Part II	**NPD**	**NPD + ACEi**	**LPD + ACEi**	**NPD + ACEi**
MAP *mm Hg*	102 ± 5	90 ± 4[a]	92 ± 6[a]	92 ± 4[a]
U_{urea} *mmol/24 hr*	415 ± 67	468 ± 64	300 ± 53[ab]	412 ± 52
U_{prot} *g/24 hr*	14.0 ± 2.1	9.3 ± 1.9[a]	6.9 ± 1.7[ab]	8.2 ± 1.7[a]

Data are mean ± SE. [a] $P < 0.05$ vs. 1st; [b] $P < 0.05$ vs. 2nd study period (ANOVA).

ACEi lowered U_{prot} with 45 ± 6% during LPD, and with 33 ± 9% during NPD. LPD decreased U_{prot} substantially less, with 17 ± 5% in patients without ACEi, and with 23 ± 7% in patients on ACEi. However, the prescribed reduction in protein intake did not always reach the desired goal, and since a correlation was found between the decrease in protein intake and U_{prot} (r = 0.85, $P < 0.001$), this suggests that a greater reduction in protein intake might result in a greater decrease in U_{prot}. Interestingly, ACEi and LPD had an additive antiproteinuric effect, as U_{prot} decreased with 51 ± 5% on combined therapy. As a result of LPD treatment GFR and ERPF decreased slightly, while serum albumin and serum protein remained stable. During ACEi treatment GFR also decreased slightly, while serum albumin, serum protein and ERPF increased significantly. In conclusion, both ACEi and LPD lower urinary protein excretion. Moreover, these regimens have an additive antiproteinuric effect. Whether this will result in an additive protective effect on the rate of renal function decline has yet to be proven.

Chlorambucil is superior to pulse cyclophosphamide in the treatment of patients with membranous nephropathy and deteriorating renal function. *L.J.M. Reichert, R.A.P. Koene, J.F.M. Wetzels, and The Study Group, Dept. of Medicine, Div. of Nephrology, University Hospital Nijmegen, Nijmegen, The Netherlands.* We examined whether immunosuppressive treatment in patients with membranous nephropathy and deteriorating renal function resulted in the preservation of renal function. In a prospective randomized trial we compared chlorambucil (CH; 0.15 mg/kg/day orally, months 2, 4 and 6) plus prednisone (P; 3 pulses methylprednisolone 1 g i.v., followed by oral prednisone 0.5 mg/kg/day, months 1, 3 and 5) with i.v. cyclophosphamide (CY; 750 mg/m^2, every month during 6 months) plus

methylprednisolone (M; 3 pulses of 1 g, months 1, 3 and 5). Eighteen patients with idiopathic membranous nephropathy (17 males, 1 female), a nephrotic syndrome (protein/creatinine index 9.1 ± 3.8 ± SD) and renal insufficiency (serum creatinine ≥ 150 μmol/liter) were studied. Before treatment serum creatinine had increased from 146 ± 69 to 260 ± 112 μmol/liter ($P = 0.003$) in the CH+P group and from 151 ± 87 to 217 ± 85 μmol/liter ($P = 0.016$) in the CY + M group. During the treatment period serum creatinine decreased in the CH + P group from 260 μmol/liter to 186 ± 74 μmol/liter ($P = 0.003$), and rose in the CY + M group from 217 μmol/liter to 297 ± 143 μmol/liter ($P = 0.02$). The difference between CH + P and CY + M was highly significant ($P <$ 0.0005). Serum albumin rose in both groups by 9 and 6 g/liter, respectively, and protein/creatinine ratio decreased by 2.5 and 3 g/mmol, respectively. At 12 months the difference in serum creatinine was sustained (CH + P 213 ± 71 μmol/liter, CY + M 596 ± 391 μmol/liter). At the end of follow-up (average 21 months, range 6–36 months) 1 patient in the CH + P group had reached end-stage renal failure after 36 months, whereas in the CY + M group 3 patients had reached ESRD after 12, 18 and 18 months, and 1 patient had died after 6 months. These results indicate that combined treatment with chlorambucil plus prednisone is superior to i.v. cyclophosphamide plus methylprednisolone in patients with membranous nephropathy and deteriorating renal function.